Recognition of Aminated Guests by Acyclic Cucurbiturils in Biological Conditions

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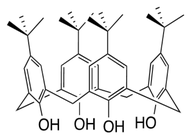
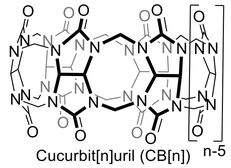
Introduction to Molecular containers

- Supramolecular chemistry is the study of covalent interactions between molecules
- In recent years, there has been growing interest in the study of molecular encapsulation particularly in the covalent intramolecular interactions between molecular containers and their guests.
- Molecular containers such as Calixarenes, cyclodextrins, and cucurbiturils have shown incredible applications, in drug delivery, molecular recognition, drug reversal, catalysis, etc.

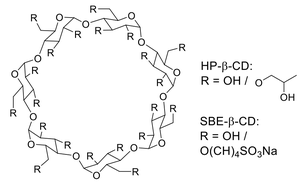


Calixarenes
- Easily functionalized
- Low binding affinity
- Small cavity
- High water solubility



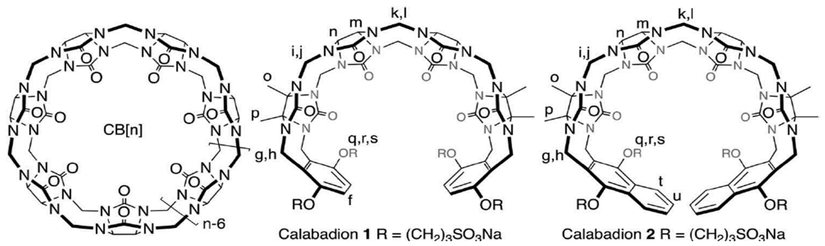
Cucurbiturils
- Difficult to functionalize
- High binding affinity
- Large cavity
- Moderate water solubility

Cyclodextrins
- Easily functionalized
- Low binding affinity
- Moderate cavity
- High water solubility



The Cucurbituril Family

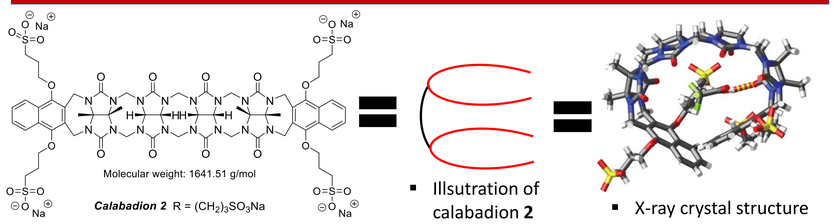
- Cucurbiturils are glycoluluril based molecular containers
- They come in two form; the cyclic cucurbiturils CB[n] and the acyclic Cucurbiturils, calabadions
- They have demonstrated a wide range of applications including as biosensors, drug encapsulation and increasing drug solubility and availability



- Cyclic , rigid structure, large cavity (12.6Å for CB[8])
- High binding affinity up to 10^{15} M^{-1}
- Low water solubility (0-30mM)
- Not easily functionalized
- Tedious and timely synthesis

- Flexible structure
- High water solubility
- Same high binding affinity
- Easier to Functionalize
- Shorter synthesis

Introduction to Acyclic Cucurbiturils



- Illsutration of calabadion **2**

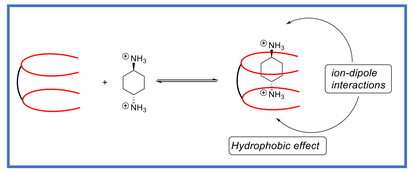
- X-ray crystal structure of calabadion **2**

- Calabadion **2** has shown strong binding affinity toward ammonium based hydrophobic guests
- **2** has been shown to enhance the solubility and bioactivity of poorly soluble pharmaceuticals [1]
- **2** has also been used as a drug reversal agent for neuromuscular blocking agents(NMBAs)[2]

Goal of the study and relevant guests

The purpose of this study was to understand the binding affinity of calabadion 2 toward several guests in 4 different environments.
- 20mM sodium phosphate buffered water, pH 7.4
- PBS (write full name)
- 35% PBS
- Fetal Bovine Protein serum

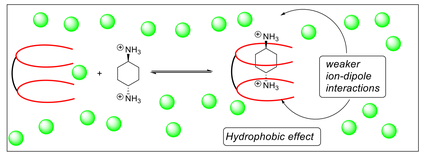
20mM sodium phosphate buffer



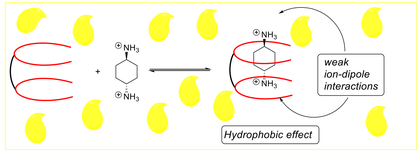
- Illustration of **2** binding to cyclohexane diammonium in 20mM Phosphate buffered water, pH 7.4
- This binding is driven by strong ion-dipole interactions between the ammonium ion and the carbonyl portal of the calabadion and the hydrophobic effect

- Illustration of **2** binding to cyclohexane diammonium in PBS buffer
- We observe the weakening of the ion-dipole interactions between the ammonium ion and the carbonyl portal because of the presence of the sodium salt

PBS buffer

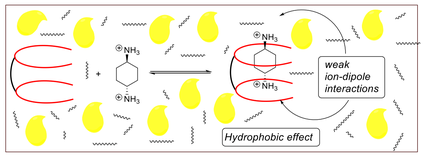


35% albumin



- Illustration of **2** binding to cyclohexane diammonium in 35% albumin
- Weaker ion-dipole interactions between the ammonium ion and the carbonyl portal
- Albumin may have some interactions with the guest or **2**

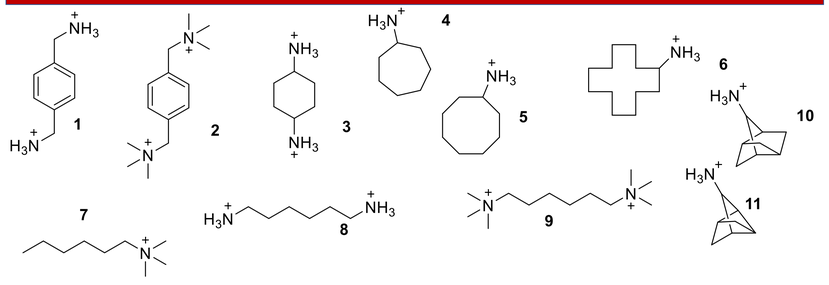
Fetal Bovine protein serum



- Illustration of **2** binding to cyclohexane diammonium in Protein serum
- Weaker ion-dipole interactions between the ammonium ion and the carbonyl portal
- Albumin may have some interactions with the guest or **2**
- Other proteins are also present

= Na^+ = other proteins = albumin

Results



Thermodynamic Data for Calabadion **2** Binding in Each Solvent of Interest

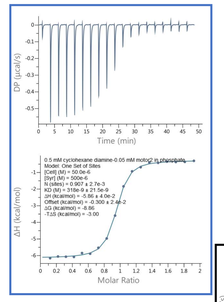
Phosphate Solvent				
Guest	Ka	ΔH	ΔG	TΔS
1	1.66×10^8	-10.9	-11.2	-0.319
2	1.42×10^{10}	-17.2	-13.8	3.34
3	3.09×10^8	-5.86	-8.86	-3
4	1.34×10^7	-8.32	-9.71	-1.4
5	3.28×10^7	-9.09	-10.2	-1.15
6	1.06×10^8	-7.67	-8.34	-0.672
7	1.75×10^7	-10.1	-9.9	0.192
8	4.30×10^7	-7.35	-10.5	-3.11
9	8.48×10^8	-9.93	-12.2	-2.3
10	3.32×10^7	-7.54	-10.3	-2.74
11	2.41×10^7	-6.15	-10.1	-3.96

PBS Solvent				
Guest	Ka	ΔH	ΔG	TΔS
1	3.00×10^7	-8.99	-10.2	-1.2
2	2.51×10^9	-15.5	-12.8	2.69
3	4.44×10^5	-4.8	-7.64	-2.84
4	3.08×10^6	-7.58	-8.86	-1.28
5	6.61×10^6	-8.7	-9.2	-0.497
6	1.09×10^6	-9.52	-6.44	3.08
7	1.74×10^7	-9.75	-8.95	0.795
8	4.30×10^7	-7.08	-9.12	-2.04
9	8.48×10^8	-9.39	-10.7	-1.27
10	3.32×10^7	-6.91	-9.27	-2.37
11	2.41×10^7	-7.13	-7.95	-0.824

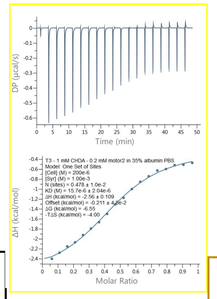
Albumin Solvent				
Guest	Ka	ΔH	ΔG	TΔS
1	6.51×10^6	-6.94	-9.32	-2.38
2	4.84×10^8	-12.9	-11.8	1.1
3	4.44×10^5	-2.5	-6.6	-4.09
4	4.76×10^5	-3.89	-7.74	-3.85
5	5.62×10^5	-5.67	-7.73	-2.06
6	3.30×10^4	-1.62	-6.09	-4.47
7	7.95×10^5	-5.78	-8.03	-2.26
8	2.67×10^5	-4.51	-7.43	-2.92
9	1.79×10^6	-6.19	-8.49	-2.31
10	4.61×10^5	-4.04	-7.74	-3.7
11	4.32×10^5	-2.73	-7.67	-4.94

Fetal Bovine Serum Solvent				
Guest	Ka	ΔH	ΔG	TΔS
1	8.07×10^7	-2.87	-6.58	-3.7
2	3.20×10^4	-6.54	-10.8	-4.26
3	3.38×10^4	5.18	-6.11	-11.3
4	2.81×10^5	2.45	-7.45	-9.89
7	6.99×10^3	-3.05	-5.27	-2.22
9	2.20×10^4	-2.43	-5.99	-3.57

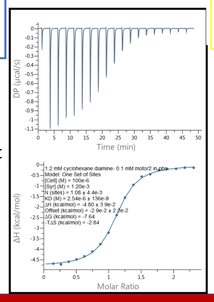
Isothermal Titration Calorimetry of calabadion 2• cyclohexane diammonium



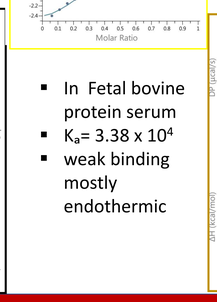
- In 20mM phosphate buffered water, pH 7.4
- K_a= 3.09 x 10^6
- Very strong binding



- In 35% albumin
- K_a= 7.07 x 10^4
- Moderate binding strength

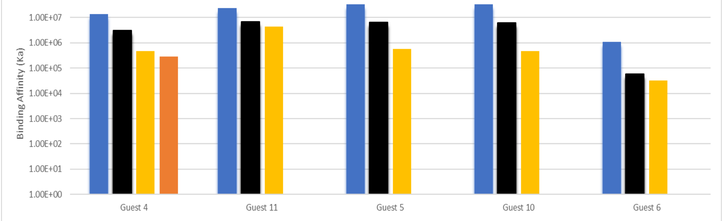


- In PBS
- K_a= 4.44 x 10^5
- Strong binding but weaker than in phosphate buffer



- In Fetal bovine protein serum
- K_a= 3.38 x 10^4
- weak binding mostly endothermic

The influence of the environment



- We observe a systematic decrease in binding affinity from the phosphate buffer to PBS to Albumin to FBS
- However, the reduction of binding affinity is quite moderate thereby indicating that Calabadion **2** still retains relatively high binding affinity in biological conditions (FBS)

Conclusion and Future Work

- The future directions of this project would first and foremost be to finish testing the Calabadion **2** binding constant with cyclic monoamines in protein serum to verify the existing data.
- The next step would be to attempt this experiment again with a different cucurbituril, possibly CB[7] to establish if these trends are unique to Calabadion **2**

Acknowledgements

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References

1. Ma, D.; Hettiarachchi, G.; Nguyen, D.; Zhang, B.; Wittenberg, J. B.; Zavalij, P. Y.; Briken,V.; Isaacs, L. *Nat. Chem*. **2012**, 4, 503-510
2. Ma, D., Zhang, B., Hoffmann, U., Sundrup, M. G., Eikermann, M., & Isaacs, L. *Angewandte Chemie International Edition.* **2012** *51*(45), 11358-11362.